

The name "N-2-hydroxyethyl-piperazine-N-2-ethanesulfonic acid" on page 13, lines 13-14 and page 20, line 16-17 has been replaced with N-(2-hydroxyethyl)-piperazine-N'-(2-ethanesulfonic acid). The amended name is in accord with the acronym "HEPES" on page 20, line 18. Support for the amended name may be found at page 1039 of the Sigma<sup>®</sup> catalog, 2002-2003 edition (attached hereto).

The name "N,N-bis-(hydroxyethyl)-2-aminoethanesulfonic acid" on page 13, lines 14-15 and page 20, line 15-16 is replaced with N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid. The amended name is in accord with the acronym "BES" on page 20, line 16. Support for the amended name may be found at page 266 of the Sigma<sup>®</sup> catalog, 2002-2003 edition (attached hereto).

The name "2-(N-morpholino)-2-hydroxypropanesulfonic acid" on page 13, lines 11-12 and page 20, line 13-14 has been replaced with 3-(N-morpholino)-2-hydroxypropanesulfonic acid. As is known to persons skilled in the art, the amended name is in accord with the acronym "MOPSO", found on page 20, line 14. Support for the amended name may be found at page 1440 of the Sigma<sup>®</sup> catalog, 2002-2003 edition (attached hereto).

Applicants respectfully submit that no new matter has been added by the foregoing clarifications to nomenclature.

Amendments to the claims

Applicants herein cancel pending claims 1-22. New claims 23-66 are added by amendment herein more particularly to point out and distinctly claim applicants' invention. Support for new claims 23-66 can be found throughout the specification, and particularly as set forth below. No new matter has been added.

Support for organic amines, as recited in new claims 23, 29, 40, 45, 50 and 56, is found particularly on page 12, lines 22 to page 13, line 6.

Support for gel buffers comprising organic amines titrated with acids, as recited in new claims 23, 25-28, 40-44 and 50-55, is found particularly on page 12, lines 22 to page 13, line 24.

Support for continuous buffer gel electrophoresis, as recited in new claims 24 and 51, is found particularly on page 11, line 31 to page 12, line 5.

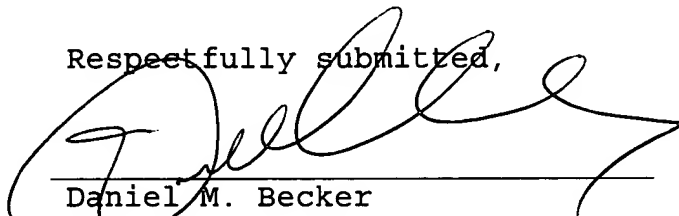
Support for an electrophoresis gel comprising agarose, as recited in new claims 23, 40 and 50, is found particularly on page 12, line 30.

Support for denaturing agents such as urea and formamide, as recited in new claims 30-32, 46-48, and 57-59, is found particularly on page 10, line 3, page 12, lines 5-11 and page 14, lines 17-21.

Support for gel buffers comprising ethylenediaminetetraacetic acid, as recited in new claims 33, 49 and 60, is found particularly on page 16, line 10, page 23, Example 8 and page 25-26, Example 13.

Support for anode and cathode buffers, as recited in new claims 34-49, 51 and 61-66, is found particularly on page 14, line 23 to page 15, line 31.

Respectfully submitted,



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**SPECIFICATION AMENDMENTS**  
**MARKED UP PURSUANT TO 37 C.F.R. § 1.121(b) (iii)**

On page 1:

This application is a continuation of copending application serial no. 09/228,875, now U.S. Patent No. 6,162,338, which is a continuation-in-part of application serial no. 08/730,678, now U.S. Patent No. 5,922,185, which is a continuation-in-part of application serial number 08/221,939, filed March 31, 1994, now U.S. Patent No. 5,578,180.

On pages 12-13:

In an embodiment of this gel and buffer system an electrophoresis gel is uniformly saturated with a gel buffer solution comprising a primary organic amine or substituted amine with a pKa near neutrality, titrated with approximately an equimolar amount of acid or zwitterionic compound, so that the pH of the buffer is between about pH 6 and pH 8, preferably between about pH 6.5 to pH 7.5, and most preferably 6.5 to 7.0. The electrophoresis gel may be any agarose or polyacrylamide gel. Preferably, the electrophoresis gel comprises between 3% and 25% (%T) acrylamide polymerized using from about 1% to about 6% cross linker (%C). More preferably, this polyacrylamide gel is polymerized using from about 2% to about 5% crosslinker (%C). Preferably, the amine comprises Bis-Tris or N-(2-hydroxyethyl) morpholine, and most preferably, Bis-Tris. Suitable acids and

61. (Newly added) The method of claim 51, wherein said electrophoresis gel is in electrical contact with an anode buffer.
62. (Newly added) The method of claim 61, wherein said anode buffer comprises tris(hydroxymethyl)aminomethane.
63. (Newly added) The method of claim 61, wherein said anode buffer comprises hydrochloric acid.
64. (Newly added) The method of claim 51, wherein said electrophoresis gel is in electrical contact with a cathode buffer.
65. (Newly added) The method of claim 64, wherein said cathode buffer comprises tris(hydroxymethyl)aminomethane.
66. (Newly added) The method of claim 64, wherein said cathode buffer comprises sodium hydroxide.

zwitterionic compounds are hydrochloric acid, tricine, acetic acid, {piperazin -N,N'-2- thanesulfonic acid} piperazine-N,N'-bis(2-ethanesulfonic acid), 3-(N-morpholino)-propanesulfonic acid, 2-(N-morpholino)-ethanesulfonic acid, N-(2-acetamido)-2-aminoethanesulfonic acid, {2-(N-morpholino)-2-hydroxypropanesulfonic acid} 3-(N-morpholino)-2-hydroxypropanesulfonic acid, {N-tris-(hydroxymethyl)-2-ethanesulfonic acid} N-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid, {N-2-hydroxyethyl-piperazine-N-2-ethanesulfonic acid} N-(2-hydroxyethyl)-piperazine-N'-(2-ethanesulfonic acid), {N,N-bis-(hydroxyethyl)-2-aminoethanesulfonic acid} N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid, and 3-(N-tris-(hydroxymethyl) methylamino)-2-hydroxypropanesulfonic acid. Tricine, 2-(N-morpholino)-ethanesulfonic acid, and {piperazine-N,N'-2-ethanesulfonic acid} piperazine-N,N'-bis(2-ethanesulfonic acid) are preferred for use in the buffer for a continuous gel and buffer system for separation of DNA and RNA because the resulting system has separation characteristics similar to the commonly used TBE gel systems. Tricine is most preferred for that use. Preferably, the gel buffer comprises Bis-Tris titrated with tricine.

On page 16:

Tris, Bis-Tris, MES, tricine, MOPS and {Piperazine-N,N'-2-ethanesulfonic acid} Piperazine-N,N'-bis(2-ethanesulfonic acid) (PIPES) were purchased from Sigma (St. Louis, MO) or Research Organics (Cleveland,

Ohio). Thioglycolic acid (TGA), dithiothreitol (DTT) and beta-mercaptoethanol (BME) were from Sigma. All other chemicals were reagent, "ultra pure" or "electrophoresis grade" from standard sources.

On page 20:

Although MES and MOPS were selected as desirable running buffers for protein separation because the resulting system has separation characteristics similar to the commonly used Laemmli and Schaeffer gel systems, it was found that a range of buffers are suitable for use in this system. Among the additional buffers giving good results were [N-(2-acetamido)]-2-aminoethanesulfonic acid (ACES), {2-[N-morpholino]-2-hydroxypropanesulfonic acid} 3-[N-morpholino]-2-hydroxypropanesulfonic acid (MOPSO), {N-Tris-(hydroxymethyl)-2-ethanesulfonic acid} N-[Tris-(hydroxymethyl)methyl]-2-aminoethanesulfonic acid (TES), {N,N-bis-(hydroxyethyl)-2-aminoethanesulfonic acid} N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES), {N-2-hydroxyethyl-piperazine-N-2-ethanesulfonic acid} N-(2-hydroxyethyl)-piperazine-N'-(2-ethanesulfonic acid) (HEPES), and 3-(N-Tris-(hydroxymethyl) methylamino)-2-hydroxypropanesulfonic acid (TAPSO).